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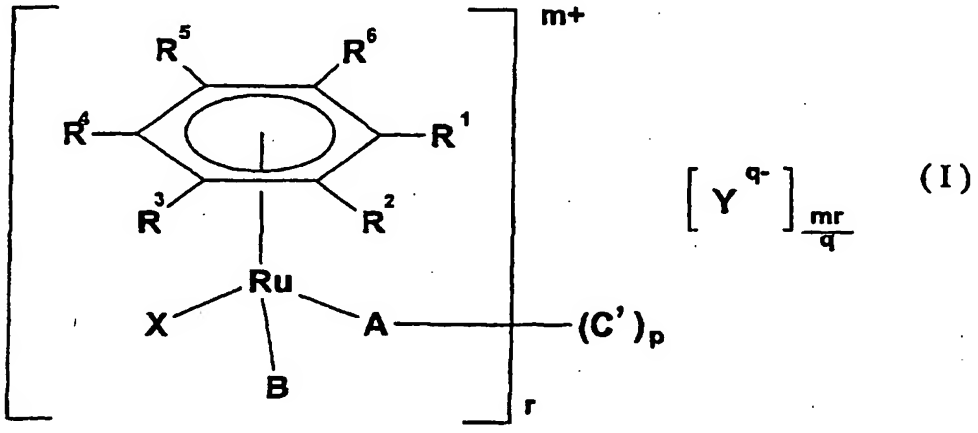
(54) Title: RUTHENIUM (II) COMPOUNDS FOR USE IN THE THERAPY OF CANCER



$$\left[Y^{q-} \right]_{\frac{mr}{q}} \qquad (I)$$

(57) Abstract: Compounds which may be used in the treatment and/or prevention of cancer have the formula (I): wherein R^1 and R^2 together with the ring to which they are bound represent a saturated or unsaturated carbocyclic or heterocyclic group.

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RUTHENIUM (II) COMPOUNDS FOR USE IN THE THERAPY OF CANCER

This invention relates to ruthenium (II) compounds, to their use in medicine, particularly for the treatment and/or prevention of cancer, and to a process for their preparation.

Certain ruthenium (II) complexes have been proposed for use in treating cancer. For example, US 4980473 discloses 1,10-phenanthroline complexes of ruthenium (II) and cobalt (III) which are said to be useful for the treatment of tumour cells in a subject.

Some other ruthenium (II) and ruthenium (III) complexes which have been shown to exhibit antitumour activity are mentioned in Guo *et al*, Inorganica Chimica Acta, 273 (1998), 1-7, specifically *trans*-$[RuCl_2(DMSO)_4]$, *trans*-$[RuCl_2(imidazole)_2]$- and *trans*-$[RuCl_4(indazole)_2]^-$. Guo *et al* discloses that the most interesting feature of these complexes is their anti-metastatic activity. Clarke *et al* have reviewed the anticancer, and in particular the antimetastatic, activity of ruthenium complexes: Chem. Rev. 1999, **99**, 2511-2533. Also, Sava has reviewed the antimetastatic activity in "Metal Compounds in Cancer Therapy" Ed by S P Fricker, Chapman and Hall, London 1994, p. 65-91.

Dale *et al*, Anti-Cancer Drug Design (1992), 7, 3-14, describes a metronidazole complex of ruthenium (II) ie, $[(\eta^6\text{-}C_6H_6)RuCl_2(metronidazole)]$ and its effect on DNA and on *E. coli* growth rates. Metronidazole sensitises hypoxic tumour cells to radiation and appears to be an essential element of the complexes of Dale *et al*. There

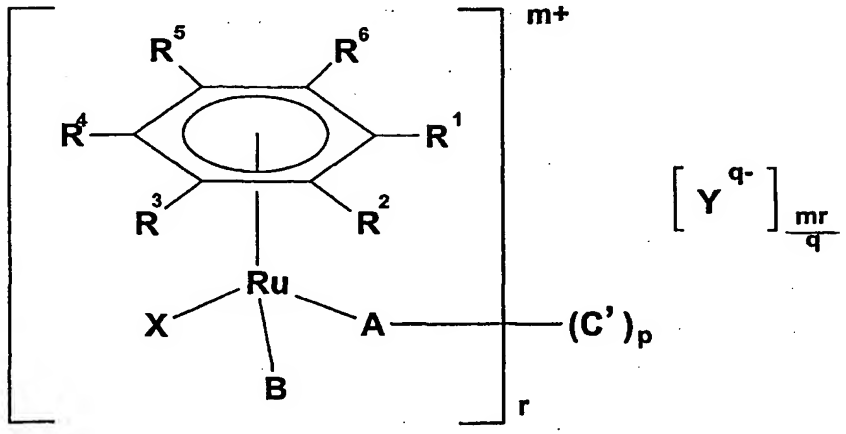
is no indication in Dale *et al* that the complexes would be at all effective in the absence of the metronidazole ligand.

Krämer *et al, Chem Eur J.*, **1996**, 2, No. 12, p. 1518-1526 discloses half sandwich complexes of ruthenium with amino esters.

There exists a need for novel anti-cancer compounds which can be used as alternatives to the compounds which are currently available.

The present invention provides a novel class of ruthenium (II) complexes having anti-tumour activity.

According to the present invention there is provided a ruthenium (II) compound of formula (I):



(I)

wherein R^1 and R^2 together with the ring to which they are bound represent a saturated or unsaturated carbocyclic or heterocyclic group containing up to three 3- to 8- membered carbocyclic or heterocyclic rings, wherein each carbocyclic or heterocyclic ring may be fused to one or more other carbocyclic or heterocyclic rings; and wherein each of the rings may be optionally substituted by one or more groups independently selected from alkyl, aryl, alkaryl, halo, carboxy, carboxyester, carboxyamide, sulfonate, sulfonamido or alkether;

R^3, R^4, R^5 and R^6 independently represent H, alkyl, $-CO_2R'$, aryl or alkaryl, which latter two groups are optionally substituted on the aromatic ring;

R' represents alkyl, aryl or alkaryl;

X is halo, H_2O, (R') (R'') S(O), R' CO_2^- or (R') (R'')C=O, where R'' represents alkyl, aryl or alkaryl and R' is as defined above;

Y is a counterion;

m is 0 or 1;

q is 1, 2 or 3;

C' is C_1 to C_{12} alkylene, optionally substituted in or on the alkylene chain, bound to two A groups;

p is 0 or 1 and r is 1 when p is 0 and r is 2 when p is 1; and

A and B are each independently O-donor, N-donor or S-donor ligands and one of A and B may be halo.

Suitably, A and B are each independently N-donor nitrile ligands; or B is halo and A is an N-donor pyridine ligand, optionally substituted at one or more of the carbon rings of the pyridine ring; or B is halo and A is an O-donor carboxylate ligand; or B is halo and A is an S-donor sulfonyl

ligand; or p is 0, A is NR^7R^8 and B is NR^9R^{10}, wherein R^7, R^8, R^9 and R^{10} independently represent H or alkyl, and A and B are linked by an alkylene chain, optionally substituted in or on the alkylene chain; or p is 1, A is NR^7 and B is NR^9R^{10}, wherein R^7, R^9 and R^{10} are as previously defined, and A and B are linked by an alkylene chain, optionally substituted.

The compounds of the invention may be in the form of solvates and/or prodrugs. Prodrugs are variants of the compounds of the invention which can be converted to compounds of formula (I) *in vivo*.

The compounds of formula (I) may have one or more chiral centres. When the compounds of formula (I) have one or more chiral centres, they may be in the form of one enantiomer, may be enriched in one enantiomer or may be a racemic mixture.

The term "alkyl" as used herein includes C_1 to C_6 alkyl groups which may be branched or unbranched and may be open chain or, when they are C_3 to C_6 groups, cyclic. Unbranched open chain alkyl groups include, for example, methyl, ethyl, propyl, butyl, pentyl and hexyl. Branched open chain alkyl groups include, for example, 2-propyl, 2-butyl and 2-(2-methyl)propyl. Cyclic groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The alkyl groups in the compounds of the invention may optionally be substituted. Substituents include one or more further alkyl groups and/or one or more further substituents, such as, for example, cyano, nitro, hydroxyl, haloalkyl, $-CO_2$alkyl, halo, thiol (SH), thioether (eg, S-alkyl) and sulfonate. The term "alkylene" is defined similarly to the definition of the term "alkyl" but includes C_2 to C_{12}

groups and is a divalent species with radicals separated by two or more (eg, from two to twelve) carbon atoms linked in a chain. Preferably, the alkylene groups are straight chain groups. Alkylene groups are optionally substituted in the akylene chain, preferably with one or more phenylene (eg, 1-4-phenylene) and/or -$CONR^{1a}$- groups and/or -NR^{2a}- groups, where R^{1a} and R^{2a} independently represent H, alkyl, aryl or alkaryl. Preferably, R^{1a} and R^{2a} are H or C_1 to C_3 alkyl.

The term "aryl" as used herein includes aromatic carbocyclic rings such as phenyl and naphthyl and heterocyclic rings such as pyridyl, imidazolyl, pyrrolyl and furanyl. Aryl groups may optionally be substituted with one or more substituents including, for example, alkyl, cyano, nitro, hydroxyl, haloalkyl, -CO_2alkyl, halo, thiol (SH), thioether (eg, S-alkyl) and sulfonate.

The term "alkaryl" means alkyl substituted with aryl eg, benzyl.

The term "alkether" means alkyl substituted with either -O- or -S- (eg, O-alkyl).

The term "halo" means a halogen radical selected from fluoro, chloro, bromo and iodo.

The term "haloalkyl" means alkyl substituted with one or more halo groups eg, trifluoromethyl.

The term "carboxyester" means -CO_2alkyl, -CO_2aryl, -OCOalkyl or -OCOaryl, preferably -CO_2 alkyl or -OCOalkyl.

The term "heterocyclic ring" as used herein refers to a 3-, 4-, 5-, 6-, -7, or 8- (preferably 5-, 6- or 7-) membered saturated or unsaturated ring, which may be aromatic or non-aromatic, containing from one to three heteroatoms independently selected from N,O and S, eg, indole.

The term "carbocyclic ring" as used herein refers to a saturated or unsaturated ring, which may be aromatic or non-aromatic, containing from 3 to 8 carbon atoms (preferably 5 to 7 carbon atoms) and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

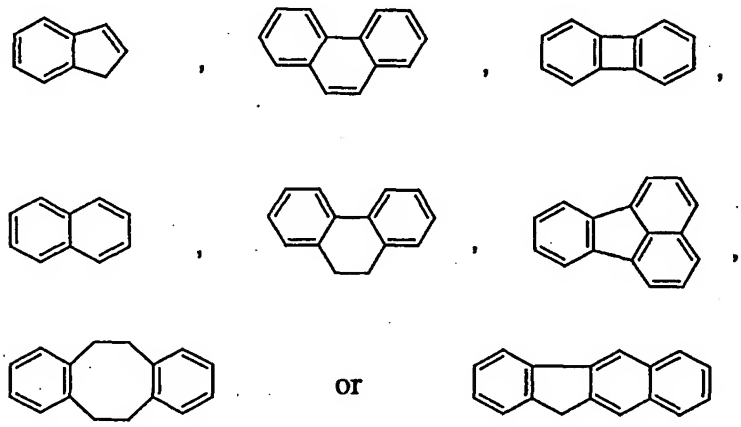
In one aspect, R^1 and R^2 together with the ring to which they are bound in compounds of formula (I) may represent an *ortho-* or *peri*-fused carbocyclic or heterocyclic ring system.

R^1 and R^2 together with the ring to which they are bound may represent a wholly carbocyclic fused ring system such as a ring system containing 2 or 3 fused carbocyclic rings eg, optionally substituted, optionally hydrogenated naphthalene or anthracene.

In another aspect, R^1 and R^2 together with the ring to which they are bound in compounds of formula (I) may represent a fused tricyclic ring such as anthracene or a mono, di, tri, tetra or higher hydrogenated derivative of anthracene. For example, R^1 and R^2 together with the ring

to which they are bound in formula (I) may represent anthracene, 1, 4-dihydroanthracene or 1, 4, 9, 10-tetrahydroanthracene.

In a further aspect, R^1 and R^2 together with the ring to which they are bound in formula (I) may represent:



In the compounds of formula (I), R^3, R^4, R^5 and R^6 may represent H.

In one aspect, A and B in the compounds of formula (I) both represent R^{11}-CN. R^{11} is alkyl, preferably C_1 to C_3 alkyl, more preferably methyl.

In another aspect, one of A and B in the compounds of formula (I) represents a $R^{12}R^{13}S(O)$ group and the other represents halo, preferably chloro. R^{12} and R^{13} are alkyl, preferably methyl.

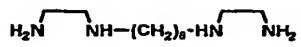
In a further aspect, A and B may together represent NR^7R^8-$(CR^{14}R^{15})_n$-NR^9R^{10}, wherein R^{14} and R^{15} are independently H or alkyl or R^{14} and R^{15}

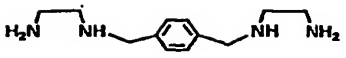
groups, on the same carbon atom or on neighbouring carbon atoms, are linked to form a carbocylic ring and n is an integer from 1 to 4. Preferably, R^{14} and R^{15} are both hydrogen and n is 2 or 3, more preferably 2. R^7, R^8, R^9 and R^{10} are preferably H or methyl and, more preferably, all of R^7, R^8, R^9 and R^{10} are H.

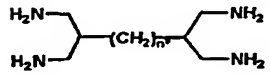
When R^8 is present in A, then p is 0. When R^8 is absent, then p is 1 and C' takes the place of R^8.

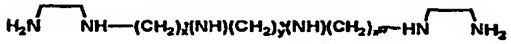
In a further aspect of the invention, R^8 is absent from A, p is 1 and C' is C_4 to C_{10} straight chain alkylene (eg hexylene). Compounds according to this aspect of the invention are so-called dinuclear complexes comprising two ruthenium atoms per complex.

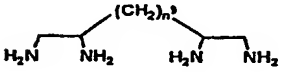
Other examples of dinuclear complexes of the invention are those in which pairs of A and B together with linker C' represent:

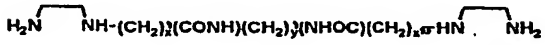


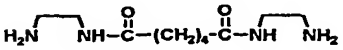


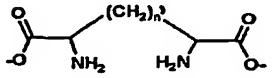


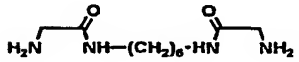


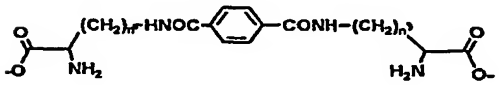












wherein each n', n'', x', x'' and y' independently represents an integer from 1 to 12, preferably 1 to 6.

Y^{q-} in compounds of formula (I) is a counterion and is only present in the compound when the complex containing the metal ion is charged. Y^{q-} is preferably a non-nucleophilic anion such as PF_6^-, for example.

R' and R'' are preferably alkyl. Most preferably, both R' and R'' are methyl.

A particular sub-group of compounds of formula I, which may be active against resistant cell lines, are those in which R_3, R_4, R_5 and R_6 are all H, R^1 and R^2 together with the phenyl ring to which they are bound form an optionally hydrogenated anthracene ring system (such as $C_{14}H_{14}$ or $C_{14}H_{12}$), X is halo, A and B are N donor ligands, p is 0, r is 1, m is 1 and Y^{q-} is a non-nucleophilic ion such as PF_6^-. Preferably, A and B are both NH_2 groups linked by a C_2-C_6 alkylene chain, more preferably a C_2 alkylene chain ie, A and B together represent ethylenediamine.

Compounds of formula (I) may be used in medicine. In particular, compounds of formula (I) may be used to treat and/or prevent cancer.

Therefore, the present invention also provides the use of a compound of the invention (ie, a compound of formula (I)) in the manufacture of a medicament for the treatment and/or prevention of cancer.

Further provided by the invention is a method of treating and/or preventing cancer which comprises administering to a subject a therapeutically effective amount of a compound of the invention.

The compounds of the invention may be used directly against a tumour. Alternatively or additionally, the compounds may be used to prevent or inhibit metastasis and/or to kill secondary tumours. It will be understood that the prevention or inhibition of metastasis is encompassed by the term "preventing cancer", as used herein.

Compounds of the invention may be effective in treating and/or preventing tumours caused by cells that are resistant to other cytotoxic drugs, such as cis-platin, for example.

The invention also provides a pharmaceutical composition comprising one or more compounds of the invention together with one or more pharmaceutically acceptable excipients. Suitable excipients include diluents and/or carriers.

The compounds of the invention may be administered by a number of routes including, for example, orally, parenterally (eg, intramuscularly, intravenously or subcutaneously), topically, nasally or via slow releasing microcarriers. Thus, suitable excipients for use in the pharmaceutical compositions of the invention include saline, sterile water, creams, ointments, solutions, gels, pastes, emulsions, lotions, oils, solid carriers and aerosols.

The compositions of the invention may be formulated in unit or sub-unit dosage form including, for example, tablets, capsules and lozenges and containers containing the composition in a form suitable for parenteral administration.

The specific dosage level of the compounds and compositions of the invention will depend upon a number of factors, including the biological activity of the specific compound used and the age, body weight and sex of the subject. It will be appreciated that the subject may be a human or a mammalian animal.

The compounds and compositions of the invention can be administered alone or in combination with other compounds. The other compounds may have a biological activity which complements the activity of the compounds of the invention eg, by enhancing its effect in killing tumours or by reducing any side-effects associated with the compounds of the invention.

The present invention also provides a process for preparing the compounds of the invention which comprises the reaction of a compound of formula $[(\eta^6\text{-}C_6(R^1)(R^2)(R^3)(R^4)(R^5)(R^6))RuX_2]$, which may be in the form of a monomer or a dimer, with A and B, optionally in the presence of Y^{q-}, in a suitable solvent for the reaction, wherein R^1, R^2, R^3, R^4, R^5, R^6, X, A, B and Y are as defined above for the compounds of the invention.

Suitable compounds of formula $[(\eta^6\text{-}C_6(R^1)(R^2)(R^3)(R^4)(R^5)(R^6))RuX_2]$ for use as starting materials (starting ruthenium complexes) in the process of the invention include $[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$, $[(\eta^6\text{-}C_{14}H_{14})RuBr_2]_2$, $[(\eta^6\text{-}C_{14}H_{14})RuI_2]_2$, $[(\eta^6\text{-}C_{14}H_{12})RuCl_2]_2$, $[(\eta^6\text{-}C_{14}H_{12})RuBr_2]_2$ and $[(\eta^6\text{-}C_{14}H_{12})RuI_2]_2$ which may be prepared according to the procedures herein disclosed.

When A and B in the compounds of the invention are R^{11}-CN, the solvent for the reaction may be R^{11}-CN itself. Preferred reaction conditions include stirring the starting ruthenium complex, as described above, in R^{11}-CN as solvent at 60°C filtering off the NH_4Cl precipitate formed and

evaporating the filtrate to yield the product. The reaction mixture comprises a source of Y^{q-}, such as a compound of formula $(NH_4^+)Y^{q-}$ eg, NH_4PF_6.

Compounds of formula (I) in which A and B represent, together, NR^7R^8-$(CR^{14}R^{15})_n$-NR^9R^{10} or NR^9R^{10}-$(CR^{14}R^{15})_n$-NR^7-C'-NR^7-$(CR^{14}R^{15})_n$-NR^9R^{10} can be produced, according to the process of the invention, by stirring the starting ruthenium complex in the presence of a slight excess of NR^7R^8-$(CR^{14}R^{15})_n$-NR^9NR^{10} or a molar equivalent amount of NR^9R^{10}-$(CR^{14}R^{15})_n$-NR^7-C'-NR^7-$(CR^{14}R^{15})_n$-NR^9R^{10}, respectively, in a suitable solvent, preferably an alcoholic solvent such as methanol. The reaction may be carried out at room temperature or at elevated temperature (eg, 30°C to 90°C) until a sufficient amount of product is formed; optionally after cooling the reaction mixture. The reaction mixture comprises a source of Y^{q-}, such as a compound of formula $(NH_4^+)Y^{q-}$ eg, NH_4PF_6.

Compounds of formula (I) in which A or B is an N-donor pyridine ligand may be obtained, according to the process of the invention, by heating a mixture of the starting ruthenium complex and excess pyridine compound (such as a 1.5- to 3- fold molar excess) in a suitable solvent such as benzene until a sufficient amount of product is formed. The reaction may be carried out under reflux conditions.

Compounds of formula (I) in which A or B is an S-donor sulfonyl ligand may be obtained, according to the process of the present invention, by dissolving the starting ruthenium complex in a solution of the sulfonyl

compound, eg, dimethyl sulfoxide, and diffusing the resulting coloured solution with a suitable solvent, eg, diethyl ether.

The precipitate which is formed in the process of the invention comprises or consists of the compound of the invention. The compound of the invention may be isolated from the reaction mixture by separating the precipitate from the liquid phase (eg, by filtration) and then removing the solvent from the precipitate (eg, under reduced pressure). The solid thus formed, which comprises or consists of the compound of the invention may, optionally, be purified eg, by recrystallisation from a suitable solvent (including, for certain compounds of the invention, acetonitrile or acetonitrile/ether (where A and B are R^{11}-CN and R^{11} is methyl) and methanol/ether).

The following non-limiting examples illustrate the present invention.

Examples

A. Synthesis

General

Ethylenediamine was freshly distilled over Na, ethanol and methanol dried over P_2O_5. Tetrahydrofuran (THF) was dried by distillation from Na-benzophenone.

Starting Materials

Preparation of 1,4,5,8,9,10-Hexahydroanthracene ($C_{14}H_{16}$) [1]

Anthracene (4.0 g, 22.4 mmol) was dissolved in freshly dried THF (200 ml) and ethanol (40 ml). This mixture was added to liquid NH_3 (500 ml) which had been condensed under argon into a 1 litre flask equipped with a Dewar condenser, cooling bath (dry-ice/acetone) and mechanical stirrer. Sodium (10.40 g, 0.45 mol) was added in small pieces over a period of 20 min. After a further 50 min stirring at -60°C, the cooling bath was removed and the ammonia was allowed to evaporate under an argon flow with stirring. Into the residue was added 50 ml water slowly to decompose the excess of sodium and then a further 150 ml. This was extracted with diethyl ether (4x250 ml) and the combined ether layers washed with saturated NaCl solution (2x250 ml) and dried over $MgSO_4$. Removal of diethyl ether on the rotary evaporator afforded the white solid. Recrystallised (2x) first from benzene-chloroform (1:1) and then from benzene only to yield a white needle product, 98% pure by ^1H NMR. This was used without further purification.

Yield: 1.54 g, 8.36 mmol, 37.3%

Preparation of 1,4,9,10-Tetrahydroanthracene ($C_{14}H_{14}$) [2]

9,10-Dihydroanthracene (5.0 g, 27.74 mmol) dissolved in 300 ml THF was added to refluxing ammonia which had been condensed under argon into a 1 litre flask equipped with a Dewar condenser, cooling bath (dry-ice/acetone) and mechanical stirrer. Li wire (0.48 g, 69.35 mmol) was added in small pieces over a period of 20 min. After refluxing for 4 h with stirring, to the reaction mixture was added 60 ml ethanol and then

120 ml water and the ammonia allowed to evaporate. This was extracted with diethyl ether (2x250 ml) and the combined ether layers washed with saturated NaCl solution (1x250 ml) and dried over $MgSO_4$. Removal of ether on the rotary evaporator afforded a light yellow solid which was recrystallized (2x) from benzene to remove most of the hexahydroanthracene ($C_{14}H_{16}$) as white needles. Further recrystallization from acetone yielded white plates of the tetrahydroanthracene ($C_{14}H_{14}$), 97% pure by ^1H NMR. This was used without further purification.

Yield: 1.5 g, 8.23 mmol, 29.7%

Preparation of $[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$ [3]

1,4,5,8,9,10-Hexahydroanthracene (1.0g, 5.43 mmol) was added to a filtered solution of $RuCl_3 3H_2O$ (0.84 g, 3.18 mmol) in dry ethanol (60 ml). The reaction was heated to reflux under argon for 48 hours. Filtration of the warm reaction mixture left a yellow-brown solid which was washed with a little ethanol, followed by diethyl ether (4x10 ml) and dried *in vacuo*.

Yield: 0.96 g, 1.36 mmol, 8.5%

Preparation of $[(\eta^6\text{-}C_{14}H_{12})RuCl_2]_2$

1,4,9,10-Tetrahydroanthracene (0.45 g, 2:49 mmol) was added to a filtered solution of $RuCl_3 3H_2O$ (0.48 g, 1.83 mmol) in dry ethanol (45 ml). The reaction was heated to reflux under argon for 48 h. Filtration of the warm reaction mixture left a brown solid which was washed with a little ethanol, followed by diethyl ether (4x10 ml) and dried *in vacuo*.

Yield: 0.57 g, 0.81 mmol, 88.5%

Example 1

Preparation of $[(\eta^6\text{-}C_{14}H_{14})RuCl(en)]^+PF_6$

$[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$(0.205 g, 0.289 mmol) was stirred in dry methanol (25 ml) under argon at 60°C. Ethylenediamine (en) (48 µl, 0.75 mmol) was added in one portion. The reaction was stirred at 60°C for 3 h and filtered and NH_4PF_6 (0.4 g, 2.45 mmol) added. The volume was reduced to approximately 6 ml on the rotary evaporator. After standing at 4°C overnight, the yellow microcrystalline solid was collected, washed with a little methanol, followed by ether and dried *in vacuo*. This was recrystallised from methanol/ether.

Yield: 0.1 g, 0.19 mmol, 32.9%

$C_{16}H_{22}ClF_6N_2PRu$(523.85) Calc. %C=36.68 %H=4.23 %N=5.35

Found %C=36.20 %H=4.17 %N=5.34

Example 2

Preparation of $[(\eta^6\text{-}C_{14}H_{14})RuCl_2(DMSO)]$ [3]

$[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$ (0.05 g, 0.07 mmol) was dissolved into dimethyl sulfoxide (2 ml) and filtered to yield a deep red solution. Slow diffusion of diethyl ether into this solution resulted in the formation of brilliant red crystals suitable for X-ray diffraction. The crystals were collected and washed thoroughly with diethyl ether (4x10 ml).

Yield: 0.03 g, 0.07 mmol, 49.5%

$C_{16}H_{20}Cl_2ORuS$(432.37) Calc. %C=44.45 %H=4.66

Found %C=44.41 %H=4.51

Example 3

Preparation of $[(\eta^6\text{-}C_{14}H_{14})RuCl(CH_3CN)_2]^+PF_6^-$

$[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$ (0.10 g, 0.144 mmol) was suspended in 10 ml acetonitrile. NH_4PF_6 (47.1 mg, 0.288 mmol) in 2 ml acetonitrile was added in one portion. The reaction was stirred at 60°C without special precautions to exclude air. After 48 h the pale brown precipitate was filtered off and orange filtrate evaporated to yield an orange solid. This was recrystallized from acetonitrile/ether to yield orange crystals.

Yield: 0.13 g, 0.238 mmol, 82.8%

$C_{18}H_{20}ClF_6N_2PRu$(545.86) Calc. %C=39.60 %H=3.69 %N=5.13

Found %C=39.17 %H=3.48 %N=5.47

Example 4

Preparation of $[\eta^6\text{-}C_{14}H_{12})RuCl(en)]^+PF_6^-$

$[(\eta^6\text{-}C_{14}H_{12})RuCl_2]_2$ (0.10 g, 0.142 mmol) was stirred in 10 ml dry methanol under argon at 60°C. Ethylenediamine (en) (24 µl, 0359 mmol) was added in one portion. The reaction was maintained at 60°C with stirring for 5 h and filtered. The volume was reduced to approximately 4 ml on the rotary evaporator and then a solution of NH_4PF_6 (0.20 g, 1.227 mmol) in 2 ml methanol was added. A yellow solid precipitated from the mixed solution when briefly shaken. After standing at 4°C overnight, this solid was collected, washed with a little methanol, followed by diethyl ether and dried *in vacuo*. This was recrystallised from benzylalcohol/ether.

Yield: 0.1g, 0.19 mmol, 67.5%

$C_{16}H_{20}ClF_6N_2PRu$(521.83) Calc. %C=36.82 %H=3.86 %N=5.37

Found %C=36.50 %H=3.85 %N=5.38

Example 5

Preparation of $[(\eta^6\text{-}C_{14}H_{12})RuCl_2(DMSO)]$

$[(\eta^6\text{-}C_{14}H_{12})RuCl_2]_2$ (0.05 g, 0.07 mmol) was dissolved into dimethyl sulfoxide (1 ml) and filtered to yield a rose red solution. Slow diffusion of diethyl ether into this solution resulted in the formation of brilliant red crystals suitable for X-ray diffraction. The crystals were collected and washed thoroughly with diethyl ether (3x10ml).

Yield: 0.025 g, 0.058 mmol, 41.4%

$C_{16}H_{18}Cl_2ORuS$(430.35) Calc. %C=44.65 %H=4.21

Found %C=44.08 %H=4.18

Example 6

Preparation of $[(\eta^6\text{-}C_{14}H_{12})RuCl(CH_3CN)_2]^+PF_6^-$

$[(\eta^6\text{-}C_{14}H_{12})RuCl_2]_2$ (0.10 g, 0.142 mmol) was suspended in 10 ml acetonitrile. NH_4PF_6 (48.6 mg, 0.298 mmol) in 2ml acetonitrile was added in one portion. The reaction was stirred at 60°C without special precautions to exclude air. After 25 h the pale brown precipitate was filtered off and the orange filtrate evaporated to yield an orange solid. This was recrystallized from acetonitrile/ether to yield orange crystals.

Yield: 0.125 g, 0.23 mmol, 81%

$C_{18}H_{18}ClF_6N_2PRu$(543.85) Calc. %C=39.75 %H=3.34 %N=5.15

Found %C=39.42 %H=3.33 %N=5.14

Example 7

Preparation of $\{[(\eta^6\text{-}C_{14}H_{14})RuCl]_2[H_2N(CH_2)_2NH(CH_2)_6NH(CH_2)_2NH_2\text{-}N,N'N'',N''']\}^{2+}.2PF_6^-$

The starting material $[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$ was prepared as previously described. Ethylenediamine and triethylamine were freshly distilled over Na. Tetrahydrofuran (THF) was dried by distillation from Na-benzophenone. Triphenylmethyl chloride (99%) and adipoyl chloride (98%) were purchased from the Arcos Chemical Co.. All other chemicals were AR grade and were used as received.

(a) N-tritylethyldiamine

A solution of trityl chloride (5.57 g, 20 mmol) in dichloromethane (25 ml) was slowly added into a solution of ethylenediamine (8 ml, 120 mmol) in dichloromethane (75 ml) with stirring at room temperature. The addition was accomplished within 1 h and the reaction stirred overnight. The white salt was filtered off and the filtrate washed with water and dried over anhydrous sodium sulphate. Dichloromethane was removed by rotary evaporation and the residue dissolved into methanol. A white precipitate began to form after shaking for a while and the mixture was kept in the refrigerator for 5 h and then filtered off. The methanol filtrate was reduced to 10 ml and kept in the refrigerator overnight. A white solid precipitated. This was collected as the desired product and washed with diethyl ether and dried *in vacuo*.

Yield: 4.5 g, 14.88 mmol, 74.4%

(b) N,N'-Bis(2'-tritylaminoethyl)-1,6-diamidohexane

N-Tritylethyldiamine (1.5 g, 4.96 mmol) and triethylamine (1.0 g, 7.29 mmol) were dissolved in chloroform (35 ml) and cooled in an ice bath. To this solution was added adipoyl chloride (0.36 ml, 2.48 mmol) in chloroform (10 ml) slowly with stirring. After addition, the mixture was

refluxed for 2 h and cooled to room temperature. This was filtered to give a clear chloroform filtrate (see below). The filtered precipitate was dissolved into dichloromethane. This was washed with water and then saturated NaCl solution and dried over anhydrous sodium sulphate. Removal of the solvent by rotary evaporation gave a white product. The chloroform filtrate was also washed with water and saturated NaCl solution and dried over anhydrous sodium sulphate. After removal of chloroform, a further crop of product was obtained.

Yield: 1.40 g, 1.91 mmol, 77%

$C_{48}H_5O_2N_4H_2O$(732.96) Calc. % C=78.66 %H=7.15 %N=7.64

Found %C=78.81 %H=6.73 %N=7.55

(c) N,N'-Bis(2'-tritylaminoethyl)-1,6-diaminohexane

To a solution of N,N'-bis(2'-tritylaminoethyl)-1,6-diamidohexane (1.3 g, 1.82 mmol) in dry THF was added a suspension of $LiAlH_4$ (0.69 g, 18.18 mmol) in dry THF (20 ml) under argon with vigorous stirring. After the addition, the reaction was heated to a gentle reflux with stirring for 25 h. This was cooled to 4°C. The reaction product and excess hydride were decomposed by the dropwise addition of H_2O (0.69 ml), followed by 15% (w/v) NaOH solution (0.69 ml) and H_2O (2.07 ml) in succession. After vigorous stirring for 30 min, the mixture was filtered by suction and the resulting cake was washed thoroughly with dichloromethane. The combined filtrate was concentrated to dryness on the rotary evaporator and the resulting residue dissolved into dichloromethane (50 ml). This was washed with water and then saturated NaCl solution and dried over anhydrous sodium sulphate. Removal of dichloromethane by rotary evaporator afforded a colourless solid.

Yield: 1.20 g, 1.75 mmol, 96%

(d) N,N'-Bis(2-aminoethyl)-1,6-diaminohexane tetrahydrochloride

A mixture of N, N'-bis(2'-tritylaminoethyl)-1,6-diaminohexane (1.0 g 1.45 mmol) and 6 M HCl (30 ml) was refluxed for 3 h. The mixture was filtered and the filtrate was concentrated to about 3 ml over *vacuo*. Addition of methanol into the concentrated solution afforded a white salt.

Yield: 0.46 g, 1.32 mmol, 92%

$C_{10}H_{26}N_4.4HCl(348.09)$ Calc. %C=34.48 %H=8.68 %N=16.09

Found %C=34.26 %H=8.77 %N=16.24

(e) $\{[(\eta^6\text{-}C_{14}H_{14})RuCl]_2$ $[H_2N(CH_2)_2NH(CH_2)_6NH(CH_2)_2NH_2\text{-}N,N',N'',N''']\}^{2+}.2PF_6^-$

$[(\eta^6\text{-}C_{14}H_{14})RuCl_2]_2$ (0.15 g, 0.213 mmol) in 10 ml methanol was stirred under argon at 60°C. To this suspension was added a solution of N,N'-bis(2-aminoethyl)-1,6-diaminehexane (0.213 mmol) in methanol which was obtained by treatment of N,N'-bis(2-aminoethyl)-1,6-diaminehexane tetrahydrochloride (73.97 g, 0.213 mmol) with 1.697 ml 0.5008 N KOH-MeOH solution. The mixture was stirred at 60°C for a further 1.5 h. This was filtered while hot and concentrated to 6 ml. Addition of NH_4PF_6 (0.25 g; 1.53 mmol) to the concentrated solution afforded a yellow precipitate. This was recrystallized from methanol/ether.

Yield: 0.09 g, 0.0796 mmol, 37.4%

$C_{38}H_{54}Cl_2F_{12}N_4P_2Ru_2(1129.85)$ Calc. %C=40.39 %H=4.81 %N=4.95

Found %C=40.30 %H=4.49 %N=4.21

B. Biological Data

1. Protocol for testing Ru compounds

The compounds are tested on 24-well trays. Cells growing in a flask are harvested just before they become confluent, counted using a haemocytometer and diluted down with media to a concentration of $1x10^4$ cells per ml. The cells are then seeded in the 24-well trays at a density of $1x10^4$ cells per well (i.e. 1ml of the diluted cell suspension is added to each well). The cells are then left to plate down and grow for 72 hours before adding the compounds of the invention.

The Ru complexes are weighed out and made up to a concentration of 1mg/ml with deionised water then sonicated until they go into solution. The appropriate volume of the Ru solution is added to 5ml of media to make it up to a concentration of 100μM for each drug. This 100μM solution is then serially diluted to make up the 10μM, 1μM and 0.1μM solutions.

The media is removed from the cells and replaced with 1ml of the media dosed with drug. Each concentration is done in duplicate. A set of control wells are left on each plate, containing media without drug.

The cells are left exposed to the drugs for 24 hours and then washed with phosphate buffered saline before fresh media is added.

They are allowed to grow on for a further 3 days before being counted using a Coulter counter.

Preparing cells for counting:

Media is removed and 1ml of PBS is added to the cells.
250µl of trypsin is added and cells left in incubator for a few minutes to allow the monolayers to detach.
Once trypsinised, 250µl of media is added to each well to neutralise the trypsin. 200µl of this suspension is added to 10ml of NaCl for counting.

2. Results

Using the above protocol, a number of compounds of the invention were tested on A2780 ovarian cancer cell line. The results are as follows:

Compound (Example No.)	IC50 (µM)
1	0.5
2	94
3	177
4	0.3
5	68
6	315
7	6

The experiments were repeated to investigate the effect of the compounds of the invention on drug-resistant variants of the A2780 cell line. The following results were obtained:

Compound (Example No.)	IC 50 (μM)		
	A2780	A2780 cis*	A2780 AD**
1	0.5	1	328
2	94	493	4
3	116	2	2
4	2	16	104
5	126	2	5
6	192	2	0.9

*Variant of A2780 showing resistance to cis-platin

**Variant of A2780 showing resistance to adriomycin. This cell line is a multidrug resistant cell line that over expresses the p glycoprotein.

Compounds of the invention therefore have cytotoxicity against cancer cells that are resistant to treatment by other drugs.

References

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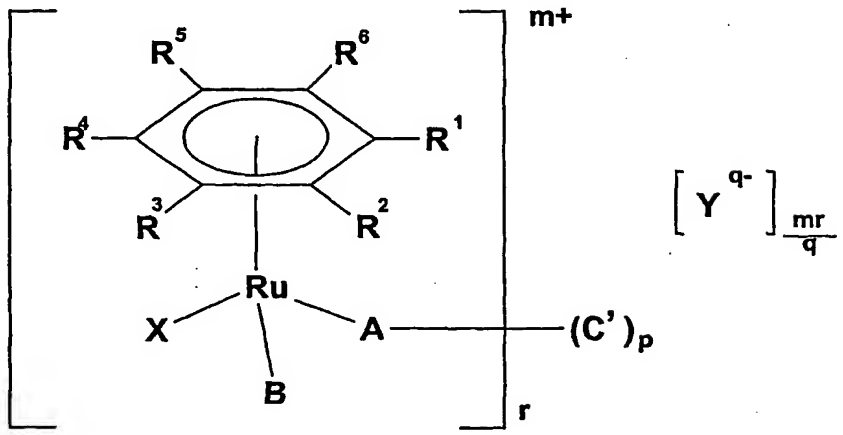
CLAIMS

1. Ruthenium (II) compound of formula (I):



(I)

wherein R^1 and R^2 together with the ring to which they are bound represent a saturated or unsaturated carbocyclic or heterocyclic group containing up to three 3- to 8- membered carbocyclic or heterocyclic rings, wherein each carbocyclic or heterocyclic ring may be fused to one or more other carbocyclic or heterocyclic rings; and wherein each of the rings may be optionally substituted by one or more groups independently selected from alkyl, aryl, alkaryl, halo, carboxy, carboxyester, carboxyamide, sulfonate, sulfonamido or alkether;

R^3, R^4, R^5 and R^6 independently represent H, alkyl, $-CO_2R'$, aryl or alkaryl, which latter two groups are optionally substituted on the aromatic ring;

R' represents alkyl, aryl or alkaryl;

X is halo, H_2O, (R') (R'') S(O), R' CO_2^- or (R') (R'')C=O, where R'' represents alkyl, aryl or alkaryl;

Y is a counterion;

m is 0 or 1;

q is 1, 2 or 3;

C' is C_1 to C_{12} alkylene, optionally substituted in or on the alkylene chain, bound to two A groups;

p is 0 or 1 and r is 1 when p is 0 and r is 2 when p is 1; and

A and B are each independently O-donor, N-donor or S-donor ligands and one of A and B may be halo.

2. Compound as claimed in Claim 1, wherein R^3, R^4, R^5 and R^6 all represent H.

3. Compound as claimed in Claim 1 or Claim 2, wherein R^1 and R^2 together with the ring to which they are bound represent anthracene.

4. Compound as claimed in Claim 3, wherein R^1 and R^2 together with the ring to which they are bound represent 1,4-dihydroanthracene.

5. Compound as claimed in Claim 3, wherein R^1 and R^2 together with the ring to which they are bound represent 1,4,9,10-tetrahydroanthracene.

6. Compound as claimed in any one of Claims 1 to 5, wherein A and B are both R^{11}-CN and R^{11} represents alkyl.

7. Compound as claimed in any one of Claims 1 to 5, wherein one of A and B is a $R^{12}R^{13}S(O)$ group and the other is halo.

8. Compound as claimed in any one of Claims 1 to 5, wherein A and B together represent NR^7R^8-$(CR^{14}R^{15})_n$-NR^9R^{10}, wherein R^{14} and R^{15} are hydrogen, or are linked at the same or neighbouring carbon atoms to form a carbocylic ring, and n is an integer from 1 to 4.

9. Compound as claimed in Claim 8, wherein R^7, R^8, R^9 and R^{10} all represent H.

10. Compound as claimed in Claim 8 or Claim 9, wherein R^{14} and R^{15} are both H and n is 2.

11. Compound as claimed in any one of Claims 8 to 10, wherein p is 0.

12. Compound as claimed in any one of Claims 8 to 10, wherein R^8 is absent, p is 1 and C' is C_4 to C_{10} straight chain alkylene.

13. Compound of any one of Claims 1 to 12 for use in medicine.

14. Use of a compound of any one of Claims 1 to 12 in the manufacture of a medicament for the treatment and/or prevention of cancer.

15. Pharmaceutical composition comprising a compound of any one of Claims 1 to 12 together with one or more pharmaceutically acceptable excipients.

16. A method of treating and/or preventing cancer which comprises administering to a subject a therapeutically effective amount of a compound of any one of Claims 1 to 12 or a composition of Claim 15.

17. Process for preparing the compound of any one of Claims 1 to 12 which comprises the reaction of a compound of formula $[(\eta^6\text{-}C_6(R^1)(R^2)(R^3)(R^4)(R^5)(R^6))RuX_2]$, optionally in the form of a dimer, with A and B, optionally in the presence of Y^{q-}, in a suitable solvent for the reaction, wherein R^1,R^2, R^3, R^4, R^5, R^6, X, A, B, q and Y are as defined in Claim 1.